THE DOGGED PATH TO ACCEPTANCE OF BORON AS A NUTRITIONALLY IMPORTANT MINERAL ELEMENT

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1. BACKGROUND BORON ESSENTIALITY STUDIES

In 1923, Warington described signs of boron deficiency for several species of leguminous plants. This was followed by a report in 1926 by Sommer and Lipman showing that boron was essential for the completion of the life cycle of a number of monocotyledonous and dicotyledonous plants. These findings probably stimulated several early eminent nutrition scientists to make attempts to show that boron was essential for higher animals; these attempts failed (Follis, 1947; Hove *et al.*, 1939; Orent-Keiles, 1941; Skinner and McHargue, 1945; Teresi *et al.*, 1944). Thus, by 1950, the dogma had developed that boron was a unique element in that it was essential for plants but not for animals; a dogma so ingrained in nutrition that it has taken almost 20 years to overcome it.

A happening in 1980 probably can be considered the seminal point in the long and vexatious path to boron being accepted as nutritionally important in higher animals including humans. After examining some growing chickens in an arsenic experiment being conducted by a graduate student, Eric Uthus, in my laboratory, I asked a quite pointed question that was preceded by a statement phrased something like: "Eric, your control chickens have abnormal looking legs and they are not growing well. Could you have left something out of the diet?" This led to the disclosure that he had made changes in the diet in an attempt to make it lower in arsenic; the changes were the

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omission of boron, fluorine and nickel from the mineral mix, and a change in the source of vitamin D.

Shortly thereafter, I asked Dr. Curtiss Hunt, a post doctorate who was working on vanadium in my laboratory at that time, to perform a simple experiment that had a profound effect on our subsequent research careers. Day-old chicks were divided into four groups and fed the modified arsenic diet supplemented with fluorine, boron, nickel, or all three elements. After three weeks, the chicks supplemented with boron grew better than those not receiving supplemental boron; neither nickel nor fluoride supplementation affected growth. The boron supplemented chicks also exhibited a more normal (but not completely normal) leg structure than the chicks supplemented with just nickel or fluoride. This led to the discovery that the new vitamin D source used in the modified arsenic diet was impotent, and to the decision to ascertain whether an interaction between boron and vitamin D affected growth and bone development in chickens.

At the Trace Elements In Man and Animals (TEMA-4) symposium in 1981, I presented some of the first findings that the collaboration between Dr. Hunt and me had produced (Hunt and Nielsen, 1981). The reception of those findings could be best described as "remarkable indifference." Attendance at the session was extremely light and questions and comments about the findings were minimal. Interestingly, in this same session, Rex Newnham gave a presentation in which he claimed boron could prevent or cure arthritis (Newnham, 1981). One of the findings I described at TEMA-4 was that rachitic long bones were found in 17 of 21 boron-deprived chicks, but only in 9 of 22 boron-supplemented chicks, fed a vitamin D deficient diet. Moreover, the lack of calcification was more severe in the boron-deprived chicks. The collaboration between Dr. Hunt and I subsequently confirmed the interaction between boron and vitamin D, and also showed that the dietary intake of calcium and magnesium affected the response to boron deprivation (Hunt, Shuler and Nielsen, 1983). In 1985, the senior scientist/post doctorate relationship between Dr. Hunt and I ended. Each of us continued to study boron independently with Dr. Hunt utilizing chicks several years before moving to rats as experimental animals, whereas I used only rats. Still, we apparently were the only scientists studying the possible essentiality of boron at this time; the reception to our findings could not be categorized as enthusiastic.

2. THE SIGNAL BORON ESSENTIALITY STUDIES BETWEEN 1987 AND 1991

Between 1987 and 1991, interest in boron essentiality was piqued beyond the Grand Forks Human Nutrition Research Center in North Dakota. In 1987, it was reported that a boron supplement of 3 mg/day markedly affected several indices of mineral metabolism of seven women consuming a magnesium low diet and five women consuming a magnesium adequate diet; the post menopausal women had consumed a conventional diet supplying about 0.25 mg of boron/day for 119 days (Nielsen *et al.*, 1987). One finding in this experiment was that boron supplementation elevated serum concentrations of 17β-estradiol and testosterone with the elevation apparently more marked in the magnesium low women. Subsequent experiments confirmed that dietary boron can affect sex steroid status in humans (Nielsen *et al.*, 1992; Samman *et al.*, 1998).

In 1987 and 1990, two additional studies performed at the Grand Forks Human Nutrition Research Center produced the most compelling findings to date (Nielsen *et al.*, 1992; Nielsen, 1989; Nielsen *et al.*, 1990; Nielsen *et al.*, 1991) indicating that boron is of

nutritional importance for humans. In these experiments men over the age of 45, post menopausal women, and postmenopausal women on estrogen therapy were fed a low boron diet or about 0.25 mg/2,000 kcal for 63 days, and then fed the same diet supplemented with 3.0 mg of boron for 49 days. Boron affected biochemical indicators related to bone turnover, physiological indicators of psychomotor and cognitive function, and blood cellular composition. For example, in both experiments, estrogen ingestion elevated serum 17 β-estradiol; this elevation was higher during boron repletion than during boron depletion. This finding indicates that boron can enhance the effects of estrogen therapy which is used to prevent bone loss in postmenopausal women. Penland (1998) found that the boron supplementation after depletion altered electroencephalograms to suggest improved behavioral activation, or less drowsiness, and mental alertness, and improved psychomotor skills and cognitive processes of attention and memory.

The suggestion that boron is a nutritionally important element based on these human findings drew mixed reactions. As bluntly stated in one review of the research: "The wisdom behind studying an element for which there is no credible evidence to indicate that it is nutritionally important can be questioned, especially when there is such a shortage of funds and opportunities to study nutritionally important elements such as zinc and iron in humans." However, these experiments expanded the interest in the possible nutritional importance of boron beyond the Grand Forks Human Nutrition Research Center. For example, Hegsted *et al.* (1991) showed that boron deprivation decreased the apparent absorption and balance of calcium, magnesium and phosphorus in the vitamin D deficient rat. King, Odom *et al.* (1991) showed that in ovo injections of boron reduced the abnormal height of long bone growth plate in chicks hatched from vitamin D deficient chickens. In 1991, the U.S. Borax company began showing an interest in supporting boron essentiality research. Their "no strings attached" support accelerated the production of conclusive findings showing that boron is not only nutritionally important but essential.

3. BORON ESSENTIALITY VALIDATION STUDIES SUBSEQUENT TO 1991

Findings brought forth since 1991 has solidified the acceptance of boron as an essential nutrient with likely practical nutritional importance. Among the more important findings has been the demonstration that the lack of dietary boron interrupts the life cycle of the frog by interfering with normal development during organogenesis and markedly impairing normal reproductive function (Fort *et al.*, 1998). Boron deficiency has been shown to have pathological consequences during two different stages of the life cycle of the zebrafish; these were membrane blebbing with cytoplasmic extrusion during the zygote and cleavage periods of embryogenesis which resulted in embryo death, and cone dystrophy in the adult stage (Eckhert and Rowe, 1999). Hunt and Idso (1999) have found that boron deprivation impairs immune function and exacerbates adjuvant-induced arthritis in rats.

The finding that the lack of boron interrupts the life cycle of some animals is adequate evidence for establishing boron as an essential nutrient for higher animals. However, significant progress is being made in establishing another criterion for essentiality; that is, a defined biochemical function. In 1991, the basis for my hypothesis that boron has a biochemical function that influences hormone action, transmembrane signaling, and/or membrane function or stability was published (Nielsen, 1991). Substantiation for this

hypothesis was findings indicating that boron deprivation affected the transport of calcium in and out of the cell (Nielsen, 1994). A fluorescent marker was used to measure cellular ionized calcium concentrations in platelets before and after activation with thrombin in the presence of external calcium; in potassium adequate rats the ionized calcium concentration upon activation was higher in platelets from boron deprived than supplemented rats. Potassium deficiency markedly reduced the ionized calcium concentration in boron deprived platelets activated with thrombin, but did not affect the concentration in platelets from boron supplemented rats. The frog and zebrafish findings described above also support the hypothesis that boron has a biochemical role at the membrane level. For example, membrane blebbing with cytoplasmic extrusion during the zygote and cleavage periods of embryogenesis, and cone dystrophy in the adult stage of zebrafish are changes occurring in cells that produce prodigious quantities of membrane (Eckhert and Rowe, 1999).

4. ACCEPTANCE OF BORON NUTRITIONALLY IMPORTANT

An analysis of both human and animal data resulted in the suggestion in a WHO/FAO/IAEA (1996) publication that an acceptable safe range of population mean intakes of boron for adults could well be 1 to 13 mg/day. Many people apparently consistently consume less than 1 mg/day, the lower value for the safe range of intakes. For example, in a group of 43 peri-menopausal women studied in the eastern North Dakota area of the United States, two women apparently consumed an average of less than 0.5 mg of boron per day, and 14 women consumed between 0.5 and 1.0 mg of boron per day (Nielsen and Penland, 1999). Rainey and Nyquist (1998) also reported that many people consistently consume less than 1 mg of boron daily.

The recent findings on boron essentiality, combined with the earlier human experiments, probably were instrumental in the decision by the Food and Nutrition Board of the National Academy of Sciences in the United States to consider establishing a Dietary Reference Intake (DRI) for boron. Based on findings to date, a DRI for boron should make people realize that consuming inadequate boron could possibly have detrimental consequences to good bone, brain, eye, immune, psychomotor, and reproductive function. In other words, all signs indicate that 20 years after boron was first suggested to be an essential nutrient for higher animals, boron will be accepted as a nutritionally important trace element in humans. At TEMA-9 in 1996, I ended my talk, which was the last one of the meeting, with the words "by the year 2000, boron most likely will be recognized as an element of clinical and nutritional importance" (Nielsen, 1997). The scientists that have labored in the dogged path to make this prediction come true should have a feeling of satisfaction for accomplishing a feat that should promote health and well-being throughout the world.

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